

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

WHAT IS CLAIMED IS:

1. A method for inducing a protective mucosal cytotoxic T lymphocyte (CTL) response in a mammalian subject comprising contacting a mucosal tissue of the subject with a composition comprising a purified soluble antigen.

2. The method of claim 1, wherein the soluble antigen is an antigenic peptide.

10 3. The method of claim 1, wherein said composition further comprises an adjuvant.

15 4. The method of claim 3, wherein the adjuvant is selected from cholera toxin (CT), mutant cholera toxin (MCT), or mutant- E. coli heat labile enterotoxin (MLT).

5. The method of claim 1, further comprising administering a purified cytokine to the subject.

20 6. The method of claim 1, wherein the cytokine is contacted with a mucosal surface of the subject.

25 7. The method of claim 5, wherein the purified cytokine is selected from granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12) or tumor necrosis factor α (TNFa).

30 8. The method of claim 1, further comprising administering purified interferon- γ to the subject.

9. The method of claim 8, wherein the purified interferon- γ is contacted with a mucosal surface of the subject.

35

10. The method of claim 5, further comprising administering purified interferon- γ to the subject.

11. The method of claim 10, wherein the purified interferon- γ is contacted with a mucosal surface of the subject.

5 12. The method of claim 1, wherein said composition further comprises a purified cytokine selected from granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12) or tumor necrosis factor.

10 13. The method of claim 1, wherein said composition further comprises purified interferon- γ .

15 14. The method of claim 12, wherein said composition further comprises purified interferon- γ .

15. The method of claim 1, wherein the antigen is a peptide derived from a pathogenic virus.

20 16. The method of claim 15, wherein the pathogenic virus is HIV-1.

25 17. The method of claim 15, wherein the pathogenic virus is influenza virus.

18. The method of claim 15, wherein the pathogenic virus is rotavirus.

30 19. The method of claim 1, wherein the antigen is a peptide derived from a pathogenic bacterium or protozoan.

20. The method of claim 1, wherein the antigen is a tumor-associated peptide.

35 21. The method of claim 1, wherein the antigen is a peptide comprising an HIV-1 cluster peptide vaccine construct (CLUVAC) selected from the group consisting of:
EQMHEDIISLWDQSLKPCVKRIQRGPGRAFVTIGK (SEQ ID NO:1),

KQIINMWQEVGKAMYAPPISGQIRRIQRGPGRAFVTIGK (SEQ ID NO:2),
RDNWRSELYKYKVVKIEPLGVAPTRIQRGPGRAFVTIGK (SEQ ID NO:3),
AVAEGTDRVIEVVQGAYRAIRHIPRRIRQGLERRIQRGPGRAFVTIGK (SEQ ID
NO:4), DRVIEVVQGAYRAIRHIPRRIRQGLERRIQRGPGRAFVTIGK (SEQ ID
5 NO:5), DRVIEVVQGAYRAIRRIQRGPGRAFVTIGK (SEQ ID NO:6),
AQGAYRAIRHIPRRIRIQRGPGRAFVTIGK (SEQ ID NO:7),
EQMHEDIISLWDQSLKPCVKRIHIGPGRAFYTTKN (SEQ ID NO:8),
KQIINMWQEVGKAMYAPPISGQIRRIHIGPGRAFYTTKN (SEQ ID NO:9),
RDNWRSELYKYKVVKIEPLGVAPTRIHIGPGRAFYTTKN (SEQ ID NO:10),
10 AVAEGTDRVIEVVQGAYRAIRHIPRRIRQGLERRIHIGPGRAFYTTKN (SEQ ID
NO:11), DRVIEVVQGAYRAIRHIPRRIRQGLERRIHIGPGRAFYTTKN (SEQ ID
NO:12), DRVIEVVQGAYRAIRRIHIGPGRAFYTTKN (SEQ ID NO:13) and
AQGAYRAIRHIPRRIRIHIIGPGRAFYTTKN (SEQ ID NO:14).

15 22. The method of claim 21, wherein the HIV-1 CLUVAC
is HIV-1 CLUVAC PCLUS3-18IIIB (SEQ ID NO:2).

20 23. The method of claim 21, wherein the HIV-1 CLUVAC
is HIV-1 CLUVAC PCLUS3-18MN (SEQ ID NO:9).

24. The method of claim 21, wherein the HIV-1 CLUVAC
is HIV-1 CLUVAC PCLUS 6.1-18MN (SEQ ID NO:12).

25 25. A method for inducing a protective mucosal CTL
response in a subject, comprising contacting a mucosal tissue
of the subject with a composition comprising a soluble
antigen, wherein said composition does not comprise an
adjuvant.

30 26. The method of claim 25, further comprising
administering a purified cytokine to the subject.

27. The method of claim 25, wherein the cytokine is
contacted with a mucosal surface of the subject.

35 28. The method of claim 27, wherein the purified
cytokine is selected from granulocyte-macrophage colony-
stimulating factor (GM-CSF), interleukin-2 (IL-2),

interleukin-7 (IL-7), interleukin-12 (IL-12) or tumor necrosis factor a (TNFa).

29. The method of claim 25, further comprising
5 administering purified interferon- γ to the subject.

30. The method of claim 29, wherein the purified
interferon- γ is contacted with a mucosal surface of the
subject.

10 31. The method of claim 26, further comprising
administering purified interferon- γ to the subject.

15 32. The method of claim 31, wherein the purified
interferon- γ is contacted with a mucosal surface of the
subject.

20 33. The method of claim 25, wherein said composition
further comprises a purified cytokine selected from
granulocyte-macrophage colony-stimulating factor (GM-CSF),
interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12
(IL-12) or tumor necrosis factor.

25 34. The method of claim 25, wherein said composition
further comprises purified interferon- γ .

35. The method of claim 33, wherein said composition
further comprises purified interferon- γ .

30 36. The method of claim 25, wherein the antigen is a
peptide derived from a pathogenic virus.

37. The method of claim 36, wherein the pathogenic
virus is HIV-1.

35 38. The method of claim 36, wherein the pathogenic
virus is influenza virus.

39. The method of claim 36, wherein the pathogenic virus is rotavirus.

5 40. The method of claim 25, wherein the antigen is a peptide derived from a pathogenic bacterium or protozoan.

41. The method of claim 25, wherein the antigen is a tumor-associated peptide.

10 42. The method of claim 25, wherein the antigen is a peptide comprising an HIV-1 cluster peptide vaccine construct (CLUVAC) selected from the group consisting of:

EQMHEIDIISLWDQSLKPCVKRIQRGPGRFTIGK (SEQ ID NO:1),

KQIINMWQEVGKAMYAPPISGQIRRIQRGPGRFTIGK (SEQ ID NO:2),

15 RDNWRSELYKYKVVKIEPLGVAPTRIQRGPGRFTIGK (SEQ ID NO:3),

AVAEGLDRVIEVVQGAYRAIRHIPRRIQGLERRIQRGPGRFTIGK (SEQ ID NO:4),

DRVIEVVQGAYRAIRHIPRRIQGLERRIQRGPGRFTIGK (SEQ ID NO:5),

DRVIEVVQGAYRAIRRIQRGPGRFTIGK (SEQ ID NO:6),

AQGAYRAIRHIPRRIQGPGRFTIGK (SEQ ID NO:7),

20 EQMHEDIISLWDQSLKPCVKRIHIGPGRFTTKN (SEQ ID NO:8),

KQIINMWQEVGKAMYAPPISGQIRRIHIGPGRFTTKN (SEQ ID NO:9),

RDNWRSELYKYKVVKIEPLGVAPTRIHIGPGRFTTKN (SEQ ID NO:10),

AVAEGLDRVIEVVQGAYRAIRHIPRRIQGLERRIHIGPGRFTTKN (SEQ ID NO:11),

DRVIEVVQGAYRAIRHIPRRIQGLERRIHIGPGRFTTKN (SEQ ID NO:12),

25 DRVIEVVQGAYRAIRRIHIGPGRFTTKN (SEQ ID NO:13) and

AQGAYRAIRHIPRRIHIGPGRFTTKN (SEQ ID NO:14).

43. The method of claim 42, wherein the HIV-1 CLUVAC is HIV-1 CLUVAC PCLUS3-18IIIB (SEQ ID NO:2).

30

44. The method of claim 42, wherein the HIV-1 CLUVAC is HIV-1 CLUVAC PCLUS3-18MN (SEQ ID NO:9).

35

45. The method of claim 42, wherein the HIV-1 CLUVAC is HIV-1 CLUVAC PCLUS 6.1-18MN (SEQ ID NO:12).

46. An immunogenic composition for inducing a protective mucosal CTL response in a subject and adapted for

intrarectal administration comprising a purified soluble antigen formulated for intrarectal delivery to the rectum, colon, sigmoid colon, or distal colon.

5 47. The immunogenic composition of claim 46, which comprises a rectal enema, foam, suppository, or topical gel.

10 48. The immunogenic composition of claim 46, further comprising a base, carrier, or absorption-promoting agent adapted for intrarectal delivery.

49. The immunogenic composition of claim 48, which includes a rectal emulsion or gel preparation.

15 50. The immunogenic composition of claim 48, wherein the soluble antigen is admixed with a homogenous gel carrier.

51. The immunogenic composition of claim 48, wherein the homogenous gel carrier is a polyoxyethylene gel.

20 52. The immunogenic composition of claim 48, wherein the soluble antigen is admixed with a rectally-compatible foam.

25 53. The immunogenic composition of claim 48, wherein the soluble antigen is formulated in a suppository.

30 54. The immunogenic composition of claim 53, wherein the suppository is comprised of a base selected from a polyethyleneglycol, witepsol H15, witepsol W35, witepsol E85, propyleneglycol dicaprylate (Sefsol 228), Miglyol810, hydroxypropylcellulose-H (HPC), or carbopol-934P (CP).

35 55. The immunogenic composition of claim 53, comprising at least two base materials.

56. The immunogenic composition of claim 46, including a stabilizing agent to minimize intrarectal degradation of the soluble antigen.

5 57. The immunogenic composition of claim 46, including an absorption-promoting agent.

10 58. The immunogenic composition of claim 57, wherein the absorption-promoting agent is selected from a surfactant, mixed micelle, enamines, nitric oxide donor, sodium salicylate, glycerol ester of acetoacetic acid, cyclodextrin or beta-cyclodextrin derivative, or medium-chain fatty acid.

15 59. The immunogenic composition of claim 46, further comprising an adjuvant.

20 60. The immunogenic composition of claim 59, wherein the adjuvant is selected from cholera toxin (CT), mutant cholera toxin (MCT), mutant- E. coli heat labile enterotoxin, or pertussis toxin.

25 61. The immunogenic composition of claim 59, wherein the adjuvant is conjugated to a mucosal tissue or T cell binding agent.

30 62. The immunogenic composition of claim 61, wherein the mucosal tissue or T cell binding agent is selected from protein A, an antibody that binds a mucosal tissue- or T-cell-specific protein, or a ligand or peptide that binds a mucosal tissue- or T-cell-specific protein.

35 63. The immunogenic composition of claim 59, wherein the adjuvant comprises a recombinant cholera toxin (CT) having a B chain of CT substituted by protein A conjugated to a CT A chain to eliminate toxicity and enhance mucosal tissue binding mediated by protein A.

64. The immunogenic composition of claim 59, wherein the adjuvant is conjugated to a protein or peptide that binds specifically to T cells.

5 65. The immunogenic composition of claim 64, wherein the protein or peptide binds to CD4 or CD8.

10 66. The immunogenic composition of claim 66, wherein the protein or peptide is an HIV V3 loop or T cell-binding peptide fragment thereof.

67. The immunogenic composition of claim 59, further comprising purified IL-12.

15 68. The immunogenic composition of claim 59, further comprising purified interferon- γ .

69. The immunogenic composition of claim 68, further comprising purified IL-12.